

Progress in Diagnosing Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like Episodes

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Abstract

Objective: Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is a progressive, multisystem affected mitochondrial disease associated with a number of disease-related defective genes. MELAS has unpredictable presentations and clinical course, and it can be commonly misdiagnosed as encephalitis, cerebral infarction, or brain neoplasms. This review aimed to update the diagnosis progress in MELAS, which may provide better understanding of the disease nature and help make the right diagnosis as well.

Data Sources: The data used in this review came from published peer review articles from October 1984 to October 2014, which were obtained from PubMed. The search term is “MELAS”.

Study Selection: Information selected from those reported studies is mainly based on the progress on clinical features, blood biochemistry, neuroimaging, muscle biopsy, and genetics in diagnosing MELAS.

Results: MELAS has a wide heterogeneity in genetics and clinical manifestations. The relationship between mutations and phenotypes remains unclear. Advanced serial functional magnetic resonance imaging (MRI) can provide directional information on this disease. Muscle biopsy has meaningful value in diagnosing MELAS, which shows the presence of ragged red fibers and mosaic appearance of cytochrome oxidase negative fibers. Genetic studies have reported that approximately 80% of MELAS cases are caused by the mutation m.3243A>G of the mitochondrial transfer RNA (Leu (UUR)) gene (MT-TL1).

Conclusions: MELAS involves multiple systems with variable clinical symptoms and recurrent episodes. The prognosis of MELAS patients depends on timely diagnosis. Therefore, overall diagnosis of MELAS should be based on the maternal inheritance family history, clinical manifestation, and findings from serial MRI, muscle biopsy, and genetics.

Key words: Functional Magnetic Resonance Imaging; Genetics; Mitochondria; Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis and Stroke-like Episodes; Muscle Biopsy

INTRODUCTION

Mitochondrial disease involves a group of rare disorders of mitochondrial dysfunction, which is usually the result of gene mutations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA). Because mitochondria are the main source of energy production in cells and are present in all tissues except for red blood cells, clinical features manifest typically in tissues with the highest energy requirements including skeletal muscles, brain, myocardium, and endocrine systems. The clinical presentation is highly variable on the disease onset, symptoms, signs, severity, and prognosis. It is common for mitochondrial disease to present with constellations of symptoms, which allows them to be categorized into one of the several syndromes.^[1]

Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is a progressive neurodegenerative disorder associated with polygenic, maternally inherited mutations in mtDNA. The disease was first reported by Pavlakis *et al.* in 1984.^[2] Approximately 80% of MELAS cases are caused by the mutation m.3243A>G of the mitochondrial transfer RNA (tRNA) (Leu (UUR)) gene (MT-TL1).^[3] The frequency of this mutation in the general population is approximately 1:15,000.^[4] The phenotypic expression of this mutation is also varied.^[5] Thus, the genetic diversity and the unpredictable presentation and clinical course highlights the difficulties in diagnosing MELAS. Promisingly, some new research progresses have been made recently, including the characteristic functional magnetic resonance imaging (fMRI) imaging, such as magnetic resonance spectroscopy (MRS), which can be used to determine the lactate level of brain, and proton

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magnetic resonance spectroscopic imaging (1H-MRSI) assessment can be used to predict disease severity. Be aware that other diseases may have similar muscle biopsy findings of mitochondrial myopathies (MM), including ragged red fibers (RRFs) and cytochrome oxidase (COX) negative fibers. The genetic test of mtDNA sequence will give the precise diagnosis. Herein, we review and analyze the research progresses in MELAS in order to help make better diagnosis of this disease.

CLINICAL FEATURES

Clinical manifestation of MELAS is highly variable, which makes it difficult to be timely accurately diagnosed. Given that mitochondria are the common pathway for the oxidative decarboxylation of fats, amino acids, and carbohydrates, it is understandable that mitochondrial dysfunction can impair cellular energy metabolism, which can be particularly apparent during periods of superimposed metabolic stress (e.g., exercise, infection, or prolonged fasting).^[6] Disease onset can occur at any age although typically the earlier the clinical phenotypes present in life, the severer the disease develops.^[1] For example, patients with the MELAS m.3243A>G gene mutation can present with the later adult-onset deafness and diabetes or fatal encephalomyopathy with seizures and stroke-like episodes (SEs) in infancy. Less than 10% of patients with genetically confirmed MELAS present before the age of 1, whereas 60–80% are diagnosed by the age of 15. There are rare case reports in the literatures of patients presenting and being diagnosed as old as 80 years of age.^[6] The average observed age of death in MELAS patients was reported to be 19–34.5 years (range 10.2–81.8 years), with 22% of death occurring younger than 18 years.^[7]

The predominant feature of MELAS involves SEs, which commonly presents acute cortical blindness, psychiatric disorder, dementia, or paralysis. Seizures are universal features of MELAS during these SEs. A wide range of clinical features can also present, including hearing loss, myopathy, neuropathy, migraine, epilepsy, ataxia, short stature, diabetes mellitus (DM), gastrointestinal disease, and cardiomyopathy.^[8,9] Previous studies have reported a large proportion of death attributable to cardiac causes in MELAS patients.^[7] Isolated MM severely affecting respiratory muscles is an uncommon clinical spectrum of m.3243A>G mitochondrial disease.^[5]

Kaufmann *et al.* reported a prospective cohort study of 85 MELAS patients (the largest sample to date) from 35 families with the m.3243A>G mutation followed for up to 10.6 years.^[7] In that study, patients with MELAS had systemic symptoms including exercise intolerance (93%), gastrointestinal disturbance (90%), loss of hearing (70%), growth failure (40%; the presence of developmental delays and growth failure was associated with an earlier onset of MELAS),^[9] diabetes (39%), hirsutism (25%), night blindness (44%), and ptosis (41%).^[7] Further, the development symptoms of the patients consist of school

difficulties (51%), child motor delay (39%), perinatal difficulties (34%), special education (34%), and speech delay (28%).^[7]

Other studies have reported considerable diversity in the clinical phenotypes that result from pathogenic mutations, including nephrotic syndrome and an altered mental status.^[10] In 2013, a Chinese case–control study of 770 Chinese Han ethnicity DM patients and 309 healthy control individuals showed 13 (1.69%) individuals carrying the m.3243A>G mutation, which was not found in any of the healthy controls.^[11] The data suggested that the m.3243A>G mutation may be a risk for DM, especially in severe cases of the disease in the Chinese Han population.^[11]

BLOOD BIOCHEMISTRY

So far, the biomarkers of MELAS in blood are rarely identified. As a common marker of injured muscles, plasma creatine kinase levels in patients with MELAS are typically normal or slightly elevated, no more than five times the upper normal limit.^[6] As lactate acidosis is one of the cardinal signs of MELAS syndrome, the most specific laboratory data for MELAS include extremely elevated lactic acid and pyruvic acid levels in both plasma and cerebrospinal fluid (CSF),^[2,9,10] and there is evidence that high lactate level is associated with increased mortality.^[7] Chinnery proposed that fasting blood lactate level above 3 mmol/L, or fasting CSF lactate level above 1.5 mmol/L supports a diagnosis of mitochondrial disease.^[12] Elevated CSF lactate level is a reliable marker pointing to mitochondrial origin of disease, even in children who have recently suffered short-lasting seizures.^[13] However, normal plasma or CSF lactate level does not exclude the presence of a mitochondrial disorder.^[12]

NEUROIMAGING

Unlike stroke, computed tomography (CT) has limited value in revealing brain lesions in MELAS, although there may be areas of focal infarction or bilateral basal ganglia calcifications on brain CT.^[4] Nevertheless, CT is often performed as it is widely available during the acute episodes of seizures and SEs in MELAS patients. MRI is the main instrument used for the diagnosis of MELAS, along with the muscle biopsy and genetic studies. For example, during SEs, brain MRI T1WI and T2WI show signal changes similar to that of acute cerebral infarction. But serial MRI typically reveals lesions that are not restricted to arterial territories, and that migrate over time. These lesions commonly affect the occipital and parietal lobes, although the deep gray matter such as the thalamus may also be affected, likely reflecting its high metabolic demand. Further, cortical lesions observed on MRI preferentially affect the cortical ribbon, with relative sparing of the deeper white matter, again reflecting the higher metabolic demand of these regions.^[4] Diffusion-weighted imaging can also reveal a hyperintense lesion in the affected lobes. As there is restricted diffusion, there is also a reduced apparent diffusion coefficient, as seen in ischemic stroke. MR

angiography (MRA) can also be used to identify segmental stenosis. Interestingly, there is evidence that the clinical symptoms and MRI/MRA findings in some MELAS patients can improve (reversible vasoconstriction) within 4 weeks.^[10] In short, the brain lesions of MELAS in serial MRI are quite different from that of acute ischemic stroke.

Magnetic resonance spectroscopy (MRS) is a useful technology to help diagnose MELAS, as it allows assessment brain metabolites. As a result of mitochondrial dysfunction, aerobic energy transduction is impaired, hence patients with MELAS have high lactate levels in blood and CSF.^[12] In MELAS, MRS can detect a higher than normal level of lactate in parts of the brain not acutely ischemic, thereby confirming a level of metabolic derangement.^[4] These changes are accompanied by decreased N-acetyl aspartate.^[4] 1H-MRSI assessment of cerebral metabolism in m.3243A>G mutation carriers is also promising for identification of disease biomarkers and individuals at risk of developing the MELAS phenotype.^[14] 1H-MRSI data can be used to estimate lactate.^[9] Elevated central nervous system (CNS) lactate as estimated by MRSI is associated with shorter survival, confirming previous observations that elevated lactate is associated with increased disease severity.^[7,14]

Yu *et al.* reported that MRI could also show quantitative changes in oxygen extraction fraction (OEF) at different phases of SEs.^[15] The use of oxygen in the brain, especially for those brain regions involved in the disease is usually severely reduced after the episodes of MELAS. Further, OEF is significantly reduced in the brain at the acute, subacute phase and interictal phase.^[15] Increased blood flow can be found in the stroke-like lesions at the acute and subacute phase.^[15] Ikawa *et al.* also reported preclinical latent lesions in patients with MELAS by arterial spin labeling (ASL) perfusion MRI.^[16] Regional cerebral hyperperfusion is observed on ASL perfusion MRI in the preclinical phase more than 3 months before the clinical onset of SEs in three MELAS patients.^[16] These hyper-perfused areas are not detected by conventional MRI in the preclinical phase and developed into acute lesions at the clinical onset of SEs, suggesting that ASL imaging has the potential for predicting the emergence of SEs.

Positron emission tomography imaging can evaluate regional cerebral blood flow, redox energy states, and glucose metabolism in the living brain, and has been used to further elucidate the pathogenesis of acute stroke-like lesions. The acute MELAS lesions appear to have an initial increase in blood flow and glucose metabolism in response to oxidative stress.^[17] In the subacute phase, there is a decrease in blood flow and glucose use even under the condition of increased oxidative stress.^[4,17]

MUSCLE BIOPSY

A specific clinical feature of MELAS is MM, which presents as exercise intolerance and weakness. As the symptoms are the most common presentations of varied myopathy, it is

strongly advised for patients who are suspected with MELAS to accept a muscle biopsy. Muscle biopsy is very helpful in confirming the diagnosis of MELAS, which mainly presents COX negative fibers and RRF.

The muscle biopsy is typically performed from a limb muscle, such as the quadriceps femoris or deltoid. The testing should include a variety of histochemical functional assays. The major diagnostic feature is the presence of fibers deficient for COX activity (mitochondrial cytopathy), which represents poor activity of complex IV of the respiratory chain, encoded by both the mtDNA and nDNA genes.^[1] It is important to note that a low frequency of COX-deficient fibers is a normal finding in healthy aged individuals. Thus, in general, the detection of any COX-deficient fibers in individuals <50 years of age, or a higher frequency of COX-deficient fibers (>5%) at any age, is strongly suggestive of a mitochondrial disorder.^[1] The identification of COX-deficient fibers is supported by serially staining muscle for COX followed by succinate dehydrogenase (SDH) that stains for complex II, which is encoded entirely by nuclear genes.^[1] The demonstration of COX-deficient, SDH-positive muscle fibers exhibits the best sensitivity and specificity for MM.^[1] As a general rule, a mosaic appearance of COX negative fibers suggests an mtDNA mutation due to the variable degrees of heteroplasmy among muscle cells, whereas uniformly decreased COX activity suggests an nDNA mutation which would be equally present in all muscle cells.^[1]

The sub-sarcolemmal accumulation of mitochondria is a classic feature of MELAS and MM, and can be demonstrated by SDH histochemistry (so-called “ragged blue fibers”) or the modified Gomori trichrome stain so-called RRF.^[1,2,6] RRF nearly always indicate a combination defect of respiratory complexes I and IV.^[1] Again, a low frequency of RRFs (<5%) can be seen in healthy aged individuals. However, the detection of RRFs in individuals <50 years of age or >5% RRF at any age is highly suggestive of MM, although even high levels can be secondary to other pathologies such as inclusion body myositis.^[1]

Other characteristic features include the presence of strongly SDH-positive blood vessels seen in muscle of patients with MELAS harboring m.3243A>G.^[1,18] Electron microscopic (EM) examination of serial frozen sections of these biopsies showed that the smooth muscle cells of the strongly SDH-reactive blood vessels contain marked proliferation of mitochondria, characteristic of patients with MELAS. EM can also be performed on muscle specimens and demonstrate a variety of abnormalities associated with MM, although these are rarely specific for mitochondrial diseases.^[1,19] These include enlarged pleomorphic mitochondria and paracrystalline inclusions. At present EM is thought to provide minor criteria for the diagnosis of MM.^[1] However, EM may provide minor diagnostic criteria for mitochondrial disease in some patients with normal histochemistry,^[1,20] and thus may contribute additional information in selected cases. Muscle histochemistry and/

or EM may be normal even in the context of genetically proven mitochondrial syndromes,^[21] particularly in the early disease course or when the biochemical defect does not involve complex IV.

Another test available from muscle tissue is respiratory chain enzyme (RCE) analysis. This testing must be performed on either fresh or snap-frozen muscle samples. RCE is technically difficult to perform, even by specialist,^[22,23] and the results should be interpreted in the context of other examinations. Demonstrating an RCE defect is a crucial diagnostic step in patients particularly children with normal or near-normal muscle histochemistry.

GENETICS

Mitochondrial DNA is a small (16,569 bases), circular, double-stranded molecule, lacking introns and possessing only one promoter region. mtDNA gives rise to two ribosomal RNAs, 22 tRNAs, and 13 protein-encoding messenger RNAs that contribute proteins to complexes I, III, IV, and V of the oxidative phosphorylation system.^[6] During fertilization, the paternal mtDNA is lost, and consequently, the mitochondria are maternally inherited.

Each cell contains several mitochondria, and each mitochondrion contains numerous genomes, with hundreds of copies of mtDNA in every cell.^[6] In this setting, the phenomenon of genetic heteroplasmy arises, where a proportion of genomes contain a mutation. The degree of heteroplasmy affects the likelihood and severity of the disease phenotype.^[6] Consequently, a pathogenic mtDNA mutation can only cause a disease phenotype if the level of the mutation within a cell or tissue exceeds a threshold amount, which varies depending on the mutation and the tissue [Figure 1].^[6] Cell types and tissues within an individual vary in the proportion of abnormal mtDNA and a given

tissue can also have different levels over time. All of these are considered as the main pathogenesis of mitochondrial diseases caused by the pathogenic mutations of mtDNA.

Patients suspected with MELAS should be further confirmed by genetic analysis. The mitochondrial tRNA (Leu (UUR)) gene (MT-TL1) appears to be a hotspot for pathogenic mtDNA mutations.^[24] The MT-TL1 gene is located in mtDNA (base pairs 3229–3303). The phenotypic heterogeneity observed with several different mutations spanning only 59 bp of this gene is difficult to interpret.^[24] These phenotypes range from myopathy alone (3302A>G), to proximal and truncal myopathy and sudden death (3251A>G), to a disorder that often predominantly affects the CNS including the MELAS syndrome (3243A>G, 3271T>C, 3252T>C).^[24] The most common mutation (bp 3243) itself has a very variable phenotype, which has also been found in patients with progressive external ophthalmoparesis, myopathy alone, diabetes, and deafness.^[24] Approximately 80% of MELAS cases are caused by the mutation m.3243A>G of the *MT-TL1* gene,^[2,6] while 7–15% of MELAS patients have the 3271T>C mutation.^[25] A host of other mutations have also been identified in MELAS patients.^[4] Some case reports show that m. 4322G>A, 3251A>G, 3252T>C, 3256C>T, 3302A>G, 3697G>A, 3946G>A, 3949T>C, 3959G>A, 3995A>G, 12300G>A, and 13513G>A mutations also lead to MELAS.^[3,25-32] It is likely that more mutations might be found in patients with MELAS in the future.

Genetic tests should be guided based on muscle biopsy findings, and these techniques are performed in combination. Mutation load can vary among tissues (including oocytes) in an individual and within a family. As blood cells have a high replication rate and selectivity against pathogenic mtDNA abnormalities, there is a decline in mutant mtDNA in blood over time.^[9] Therefore, many mtDNA abnormalities are not detectable in blood. However, it should not be assumed

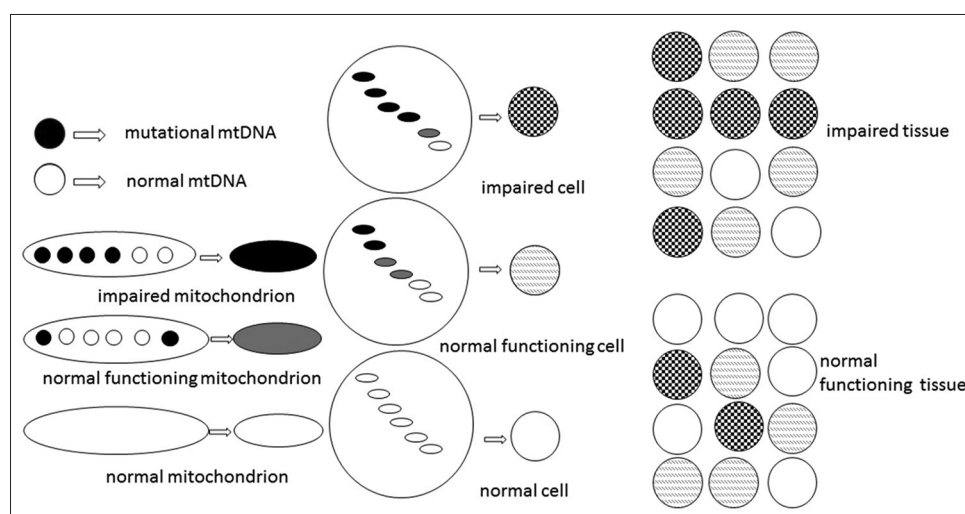


Figure 1: Mechanism of the threshold effect and heteroplasmy. Every mitochondrion has several mitochondrial DNA (mtDNA) copies. In patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, if mutational mtDNA exceeds the threshold, the mitochondrial function is impaired. If the mutation does not exceed the threshold, the mitochondrial function may be normal. Cells and tissues have the same mechanism.

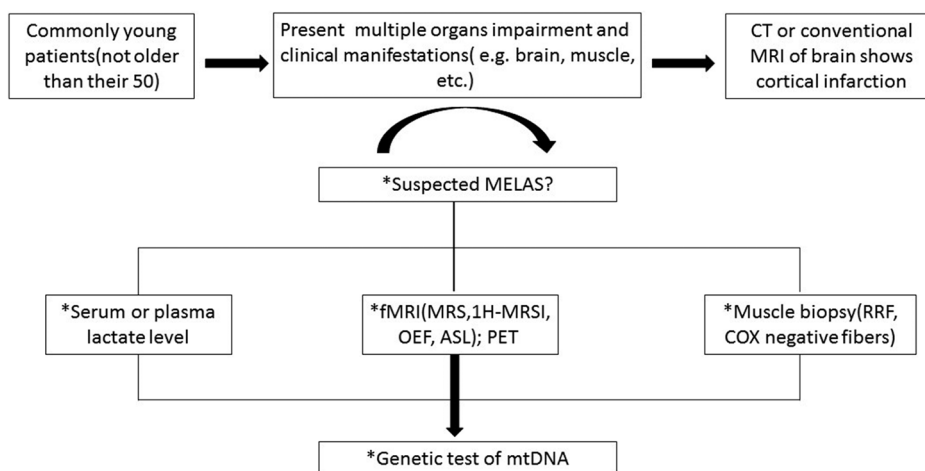


Figure 2: Diagnosis flow chart. *Represents the key elements for the right diagnosis of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes.

that mutation load is negligible, as it may be higher in other tissues.^[9] In this setting, the assessment of mtDNA should be done from muscle DNA extracts.^[1]

DIFFERENTIAL DIAGNOSIS AND THERAPY

Because MELAS is a group of rare disorders that presents with varied clinical phenotypes, it is, therefore, easy to misdiagnose. The first symptoms include exercise intolerance, seizures, paralysis, hemianopsia, mental anomaly, gastrointestinal disorders, respiratory muscles weakness, and arrhythmia.^[7-9] Patients with MELAS are usually sent to the emergency room, and are predominantly initially misdiagnosed as myasthenia gravis, epilepsy, cerebral infarction, encephalitis, gastrointestinal diseases, or heart diseases, leading to mistaken treatment and delayed therapeutic opportunity.^[1,4,7,9] Physicians should notice that patients are usually young. The plasma lactate level should be examined first, then brain CT and MRI. Elevated plasma lactate levels and lesions mainly located in the cortex, with relative sparing of the deeper white matter on CT and MRI, indicate suspected MELAS.^[15-17] Further investigation should be performed stepwise to confirm the diagnosis [Figure 2].

There is no specific treatment for MELAS. Current therapies include supplement of L-arginine, citrulline, ATP, vitamins, riboflavin, and CoQ10. Avoiding infection, fatigue, psychological trauma and prolonged fasting is also strongly recommended.^[12] The use of these methods can improve prognosis in patients with MELAS.

In conclusion, MELAS is a progressive neurodegenerative disorder associated with polygenetic, maternally inherited mutations in mtDNA. The predominant feature of MELAS is SEs, and the onset usually occurs in the second decade. High plasma and CSF lactate levels may indicate MELAS. The main diagnostic feature of muscle biopsy is the presence of RRF and mosaic appearance of COX negative fibers. Mitochondrial genetic examination

can confirm the diagnosis, the frequent mutations are 3243A>G, 3271T>C, and 3252T>C. New techniques of fMRI especially 1H-MRSI and OEF enhance the diagnosis of MELAS. The overall diagnosis should be based on not only the clinical manifestations, but also the laboratory findings from blood and CSF biochemistry, neuroimaging, muscle biopsy, and genetic examination. Special attention should be paid because of the variability in disease stages and genetic heteroplasmy. For example, muscle biopsy may be normal in the early disease course, and mtDNA abnormalities are not detectable in blood. The prognosis of MELAS patients depends on the location of genetic mutations, mutation load, and the onset of disease and interventions.

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